DAHL-CHASE Focus

Technical Bulletin: FISH for Recurrent Bladder Cancer

Technical Brief

FISH for Recurrent Bladder Cancer: Detection of Genetic Alterations in Bladder Cancer Cells

Dahl-Chase Diagnostic Services is now offering analysis of chromosomal abnormalities in urine specimens using multicolor, multi-target UroVysion FISH probes for the detection of bladder cancer recurrence.

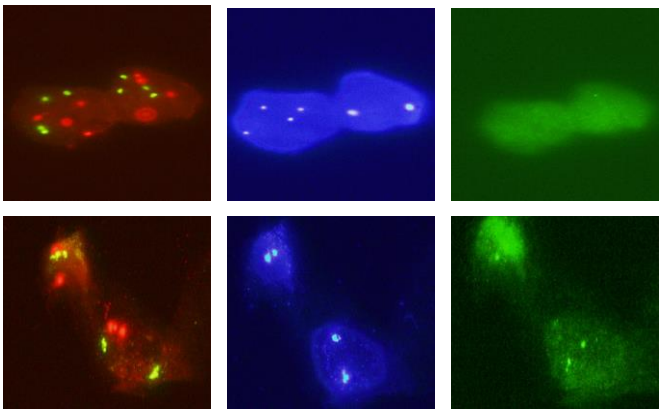
Background Information

Bladder Cancer is the fifth most common type of urothelial carcinoma in the United States. Ninety percent of all bladder cancer cases are urothelial carcinomas (UC). At presentation, about 75% of tumors are superficial, of which 50% to 80% will have one or multiple recurrences, and 15% to 25% will progress to muscle-invasive tumors. Follow-up cystoscopy and urine cytology have been used to detect recurrence and tumor progression in patients with superficial UC. However, low-grade tumors tend to have false negative cytology results.

Several genetic alterations have been identified to occur at high frequency in bladder cancer. These include the loss of a portion of chromosome 9 (presumably carrying a tumor suppressor gene), as well as aneuploidy of chromosome 3, 7, and 17 (1). Several studies have demonstrated that detection of chromosomal abnormalities by fluorescence *in situ* hybridization (FISH) has higher sensitivity in detection of UC recurrence than cytology, while maintaining high specificity (2-4). The presence of chromosomal abnormalities also has been shown to predict bladder cancer recurrence and is detectable by UroVysion (5,6). The UroVysion Bladder Cancer Kit (UroVysion, Abbott Molecular, Vysis, Des Plaines, IL) is an FDA approved in-vitro diagnostic assay used to detect aneusomy for chromosomes 3, 7, and 17 and loss of 9p21 locus.

Clinical Indications

UroVysion FISH testing is performed on persons with hematuria suspected of having bladder cancer and for subsequent monitoring of tumor recurrence in patients with previously diagnosed bladder cancer. FISH analysis of urine specimen can be utilized as an ancillary test thereby increasing the sensitivity of urine cytology for the detection of UC.



An illustrative image of UroVysion FISH from a bladder cancer patient (top row from left to right) demonstrates gains of chromosomes 3 (red), 7 (green), and 17 (aqua), and loss of 9p21 locus (gold). Nuclei of benign urothelial cells (bottom row) demonstrate two copies of each probe.

Methodology

The UroVysion Bladder Cancer Kit is an FDA approved In-Vitro Diagnostic assay designed to detect aneusomy of chromosomes 3, 7, and 17 and loss of 9p21 locus via FISH in urine specimen using a 4-color, four-probe mix of fluorescently labeled DNA probes. Urine specimen are collected and resuspended with PreservCyt (Thin Prep UroCyte Urine Collection Kit). Urine specimens are to be refrigerated and shipped on ice within 24 hrs of collection. Thin Prep UroCyte Urine Collection kits

may be obtained from Dahl-Chase Diagnostic Services (207) 941-8202.
Cells recovered from urine specimen are fixed on slides. The slides are protease digested, fixed in formaldehyde, washed, and dehydrated. The DNA is denatured to single stranded form and allowed to hybridize with the UroVysion DNA probes. Probe hybridization is performed using a DNA probe mixture containing fluorophore-labeled DNA probes to the peri-centromeric regions of chromosomes 3 (CEP3-red), 7 (CEP7-green,), and 17 (CEP17-aqua), and to the locus 9p21 (LSI 9p21-gold). Unbound and non-specifically bound probe is removed by a series of washes, and nuclei are counter stained. Morphologically abnormal nuclei are enumerated using fluorescence microscopy.

Interpretation

Morphologically atypical nuclei containing fluorescent signals for CEP 3 (red), 7 (green), and 17 (aqua) and the 9p21 locus (gold) are enumerated for minimum of 25 nuclei.

A positive result**:**
4 or more nuclei with a signal gain of 2 or more chromosomes (3, 7, and 17) in the same nucleus and/or homozygous loss of the 9p21 locus in 12 or more nuclei.

Assay Limitations

Urine specimen submitted for evaluation that are of low cellularity, low volume or any other situation that may result in a specimen containing less than 25 urothelial cells.

Test Overview

Test Name	FISH for bladder cancer
Patient Preparation	None
Reference Range	Fluorescent signal quantification of morphologically abnormal nuclei (see positive result definition above)
Specimen Requirements	A minimum of 33-60cc of fresh voided urine poured immediately into urine preservative (PreservCyt). Mix and refrigerate. Transport refrigerated. Submit with molecular requisition.
CPT Codes	88120

References

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